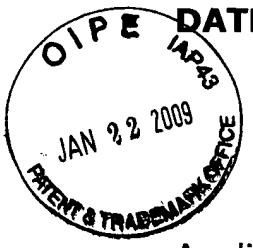
EXPRESS MAIL NO.: EV 963177026 US **PATENT**
DATE DEPOSITED: JANUARY 22, 2009



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Hildebrand et al.	)	Atty Dkt No: 66802.055
Serial No:	10/669,925	)	Examiner: M. DiBrino
Filed:	September 24, 2003	)	Art Unit: 1644
Customer No.: 30589		)	Confirmation No.: 4622

For: ANTI-HLA ASSAY AND METHODS

MAIL STOP - AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.132

I, Rico Buchli, hereby declare that I am a co-inventor of the invention claimed in the subject application, along with William H. Hildebrand, who is a co-author of the Prilliman et al. (Immunogenetics, 45:379-385 (1997)) reference. I have reviewed the purification procedure described in Prilliman et al.

The purification procedure described in Prilliman et al. is useful for the purification of peptides from a denatured HLA trimolecular complex. The purification procedure described in Prilliman et al. is not useful for the isolation of functionally intact HLA trimolecular complexes. In the purification procedure described in Prilliman et al., soluble HLA trimolecular complexes were produced in a lymphoblastoid cell line in culture, and the supernatants were subjected to affinity chromatography, wherein HLA light chain-specific antibodies (BBM.1) were coupled to the affinity column. The column elution procedure utilized in the method was acetic acid, pH 2.0. Under these conditions, the HLA trimolecular complexes were denatured. Therefore, the purification procedure described in Prilliman et al. isolates peptides but not intact HLA trimolecular complexes.

Attached hereto as Exhibit B are three experiments that I performed; these experiments demonstrate that when HLA trimolecular complexes come in contact with the acidic elution buffer utilized in Prilliman et al., namely, 0.2 N acetic acid pH 2.0, such HLA trimolecular complexes do not maintain a native structure.

In Experiment 1 shown in Exhibit B, intact sHLA trimolecular complexes were exposed to different buffers/solutions for different time periods to test the stability of the HLA trimolecular complexes. Test specimens were neutralized immediately before ELISA-testing to allow

antibody interaction at neutral pH. The results of Experiment 1 clearly illustrate that 0.2 N acetic acid does not detect any intact sHLA trimolecular complexes using the conformational antibody W6/32.

In Experiment 2, Superdex Chromatography was used to visualize sHLA proteins as native, intact complexes in PBS (Trace 1) and after treatment with 0.2N acetic acid pH 2.0 (Trace 4). A beta-2-microglobulin sample (Trace 2) was run as a positive control, and sHLA storage buffer (PBS/NaAzide pH 7.4; Trace 3) was run as negative control. The results of Experiment 2 clearly demonstrate that after acid-treatment, intact sHLA trimolecular complexes are not detectable.

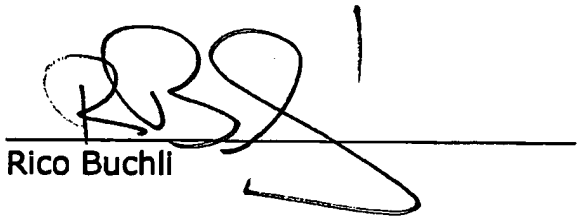
In Experiment 3, sHLA trimolecular complexes used within Experiment 2 were further tested for W6/32 activity. The antibody W6/32 recognizes intact sHLA trimolecular complexes only. The results of Experiment 3 confirm that within the detectable assay range of W6/32-sHLA interaction, no activity was observed for the acid-treated sample.

Thus, no functionally active, MHC trimolecular complexes can be purified substantially away from other proteins and maintain the physical, functional and antigenic integrity of the native MHC trimolecular complex using the method of Prilliman et al. In contrast, the methods described and claimed in the subject application utilize recombinantly produced, functionally active and intact HLA trimolecular complexes that have been

purified substantially away from other proteins.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,



Rico Buchli

EXHIBIT B

Experiment 1

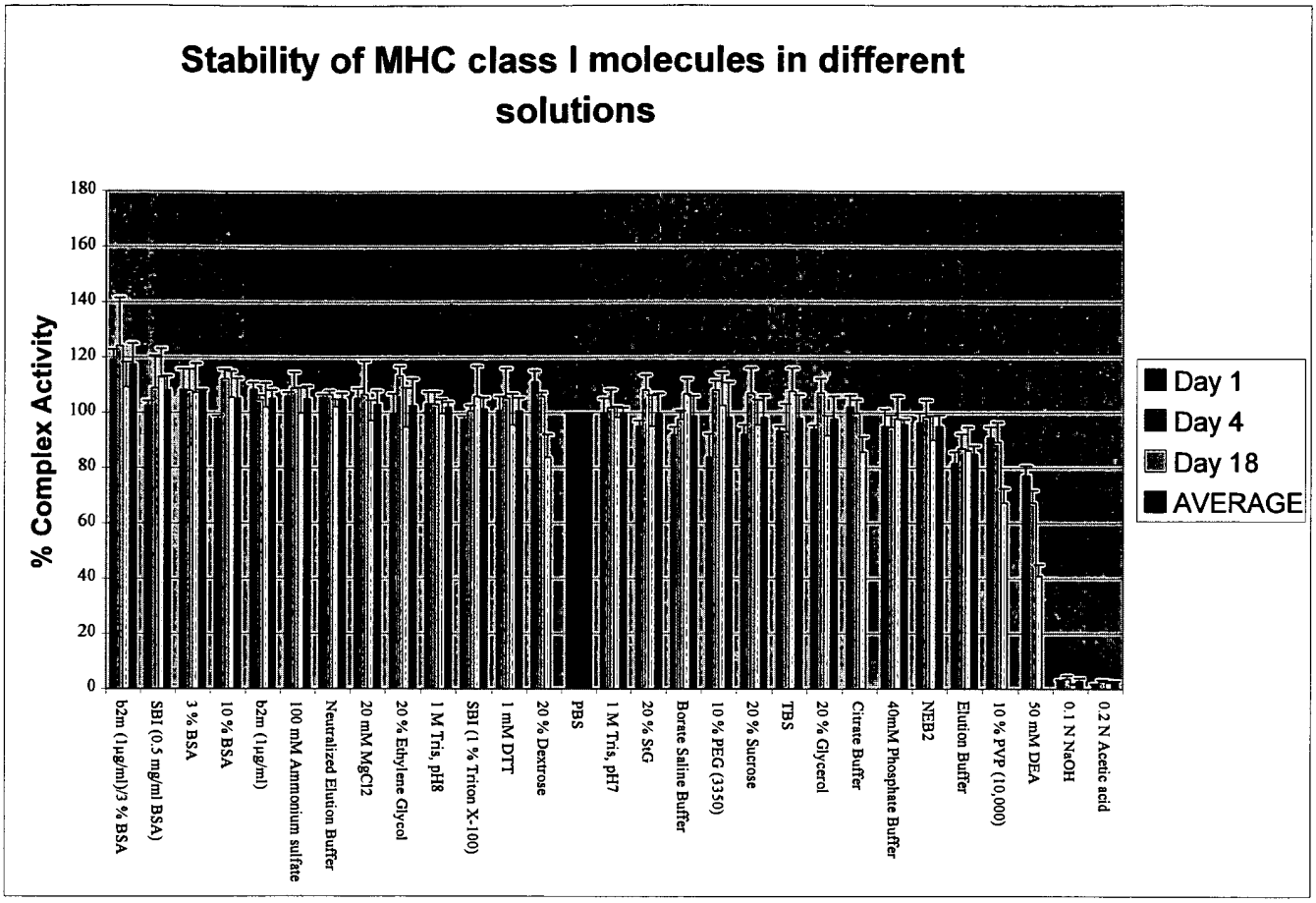


EXHIBIT B (Continued)
Experiment 2

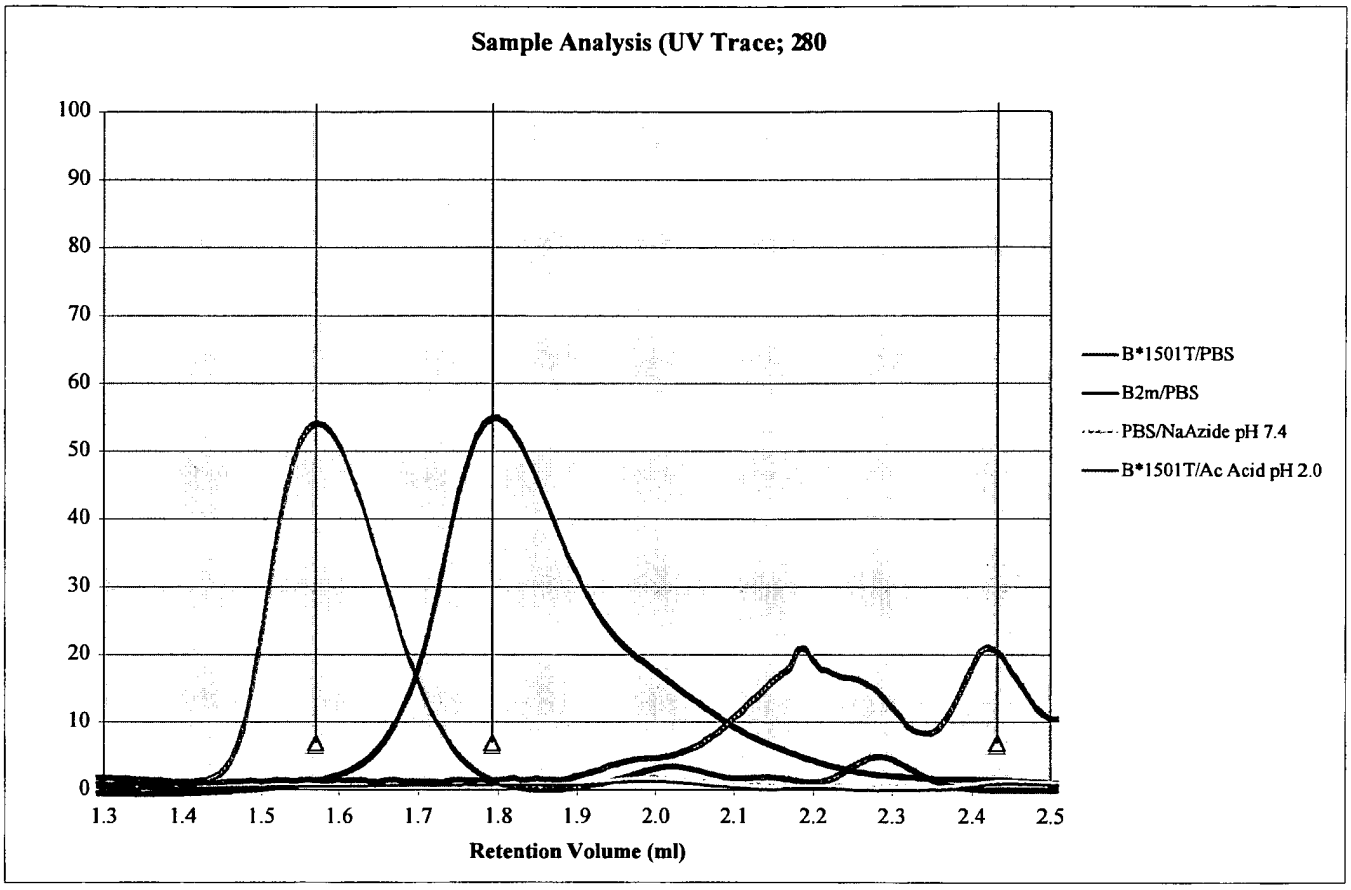


EXHIBIT B (Continued)

Experiment 3